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## Thermal gradient fractionation of glyceride mixtures under supercritical fluid conditions

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### Abstract

Supercritical CO<sub>2</sub> was used to fractionate a mixture of monoacylglycerols (MAGs), diacylglycerols (DAGs) and triacylglycerols (TAGs) using an eight-foot packed column which was kept under thermal gradient conditions and operated semi-continuously. Fractionation of the glyceride mixtures was affected by both their solubility in CO<sub>2</sub> and respective vapor pressure enhancement. The feed material used, approximating a feed stream used in the industrial enrichment of MAGs, was composed of 48.7 wt.% MAG, 46.3 wt.% DAG and 5 wt.% TAG, having main acyl chain compositions of 6 wt.% C16:0, 23 wt.% C18:0 and 59 wt.% C18:1. The resultant top product from the fractionation column had MAG concentrations as high as 90 wt.% and no TAG, which is comparable to that produced by molecular distillation.

The effects of pressure, temperature gradient, CO<sub>2</sub> flow rate and feed composition on the separation efficiency and yields were investigated. Increasing the column pressure from 172 to 344 bar or the density of CO<sub>2</sub> from 420 to 720 kg m<sup>-3</sup> increased the yield of the top product. However, with those increases, the supercritical fluid became less selective, with the MAG concentration decreasing from 84 to 56 wt.%. At 207 bar, a linear temperature gradient varying from 65 to 95°C gave the best selectivity when compared to other temperature gradient schemes. Increasing the CO<sub>2</sub> flux from 1.8 to 10.3 g min<sup>-1</sup> cm<sup>-2</sup> resulted in little change in the MAG concentration, but the top-product yield decreased from 14.4 to 2.3 g kg<sup>-1</sup> CO<sub>2</sub>. As the MAG concentration in the feed mixture increased from 48.7 to 63 wt.%, the MAG concentration in the top product (at 207 bar and 65–95°C) increased from 79.4 to 86 wt.%. © 1997 Elsevier Science B.V.

**Keywords:** Column; Fractionation; Glycerides; Supercritical fluids

### 1. Introduction

Surface-active lipids are used throughout the food, pharmaceutical and cosmetic industries to improve product performance. Such lipids have

found a wide range of applications in the food industry, where they are used for emulsification, starch complexing, aeration, as defoaming agents and as oil stabilizers. Monoacylglycerols (MAGs) of saturated and unsaturated fatty acids are the most commonly used emulsifiers, and account for over 70% of the total world use, exceeding over 200 million pounds per year [1]. The use of high-

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MAG products in the food industry has increased steadily due to the desire to use purer and better-defined products with superior performance properties. For example, in cakes and icings (frostings), the textural profile of the product in the mouth is critical, and depends on the composition of the emulsifier which is added.

Monoglycerides are partial esters of glycerol containing higher molecular-weight fatty acids. They are commonly produced by esterifying glycerol with fatty acids, by transesterification of triacylglycerols (TAGs) with glycerols, or by enzymatic cleavage of TAGs. The objective of these reactions is to obtain a product of defined glyceride composition, and frequently with maximum monoacylglycerol and minimum triacylglycerol content. However, these reactions often produce a mixture of MAGs, DAGs and TAGs. For many purposes, the equilibrium mixtures obtained from glycerolysis or transesterification reactions may be used directly following the separation of the unreacted glycerol. When this mixture is used as a food emulsifier, DAGs and TAGs are essentially inert and merely dilute the MAGs composition. Therefore, separation of glycerides to produce a high-value product which is rich in MAG is desirable.

The commercial emulsifier product mixtures are called '40% monos', '60% monos' or '90% monos', depending on the percentage of MAGs. Separation of these glyceride mixtures is based on the vapor pressure and molecular weight of their respective constituents. The commonly used separation methods for glycerides are molecular distillation, adsorption, and chromatography. High monoacylglycerol-containing emulsifiers are commonly manufactured using molecular distillation. However, the use of high vacuums and temperatures (usually more than 200°C) associated with molecular distillation are problematic, leading to the possibility of alteration of some of the components and the formation of undesired products [2,3].

A possible new refining technology for manufacturing high-MAG emulsifiers is supercritical fluid fractionation. A supercritical-fluid based process is a viable alternative to the conventional process for separating these high-value feedstocks from reaction mixtures or by-product streams. Pure or

modified supercritical carbon dioxide (SC-CO<sub>2</sub>) may be used for separation of nonpolar lipid components from polar moieties, or for the fractionation of lipid materials having significant differences in molecular weight or vapor pressure. Fractionation in a packed column amplifies these molecular differences by varying the solvent strength of the fluid through sequential pressure changes or via a longitudinal temperature gradient.

There are many reports describing the potential of SCF solvents to fractionate polymers with respect to molecular weight, chemical composition and backbone structure, and to purify speciality monomers which cannot be processed by other separation techniques [4-7]. There has been limited research on fractionation of the lipid constituents of natural oils, but studies have demonstrated the potential of thermal gradient [8-10] or pressure gradient [11], and packed-column fractionation [12] methods toward this end. The motivation for these studies has been to alter the chemical composition of the feedstock, such as fractionation and concentration of fatty acid ethyl esters or removal of terpenes from citrus oil. Fractionation studies to isolate ethyl esters of codfish oil have shown that it is possible to isolate 90% or more of the C20 esters in the retrograde region (150 bar) using an internal 'hot finger' [13]. Isolation of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic (DHA) from fish-oil derivatives [8], from herring oil [9], and anchovy oil [2] has also been demonstrated to be amenable to supercritical fluid fractionation (SFF). Fractionation using hydrocarbon solvents such as ethane, propane, butane, pentane, ethene, propene and butene at high pressures has been used to recover high-grade monoglyceride from a mixture of glycerides and glycerol [14].

In recent years, particular emphasis has been placed on the production of fractions of anhydrous milk fat using SFF [15-17]. Fractions obtained by the supercritical fluid process melt more homogeneously than those obtained by fractional crystallization. Other studies employing SFF have been made on olive oil [18], citrus oil [19,20], butter fat [21] and menhaden oil [8,22]. Most column fractionation studies to date have been semi-continuous operations, although a few large-scale con-

tinuous operations have been reported [23,24]. A mathematical model [15] has been applied to the fractionation of milk fat, and a process study and economic analysis have been provided [17].

The thermodynamic basis and the process dynamics for the column fractionation of glycerides has not been completely understood. When a mixture of glycerides is allowed to equilibrate with SC-CO<sub>2</sub>, some of the glycerides dissolve in the CO<sub>2</sub> phase while some CO<sub>2</sub> dissolves in the glyceride phase. Such a system, consisting of a glyceride-rich phase and a CO<sub>2</sub>-rich phase, may be considered as a pseudo-binary system. The distribution of glyceride species between the two phases is the basis of separation and fractionation. This distribution depends on the glycerides' vapor pressure and the CO<sub>2</sub>-glyceride and glyceride-glyceride interactions. These interactions are influenced by differences in molecular weight, polarity and chemical structure of the glyceride species. Distribution of the glyceride species between the two phases can be approximated by determining their interphase distribution coefficient. For a given mixture, the distribution coefficient is a function of solvent strength (i.e. density of CO<sub>2</sub>) and temperature. A theoretical treatment of the equilibrium relationships of a multicomponent two-phase system is complicated. The principles involved are essentially the same as a binary system, except that we have to account for solute-solute interactions.

Solubilities of triglycerides found in fats and oils have been reported by many researchers [25–29]. The solubility of monolaurin in SC-CO<sub>2</sub> was found to be 30 times lower than those for di- and trilaurin. Although trilaurin has a molecular weight 2.3 times higher and a vapor pressure 150 times smaller than monolaurin, the high polarity of monolaurin was found to limit its solubility in SC-CO<sub>2</sub> [28]. Increasing the fatty-acid chain length results in a greater solubility of MAG compared to TAG of an equivalent fatty-acid chain length, as demonstrated for the case of a monooleylglycerol/triolelylglycerol pair [30]. Therefore, as the fatty-acid chain length increases, the difference in molecular weight and vapor pressure between the MAGs, DAGs and TAGs becomes more significant, minimizing the polarity differences of the respective lipid species. Complete

solubility data for all the common mono-, di- and triglyceride species in SC-CO<sub>2</sub> is not available, and there has not been a systematic study performed to date to measure the distribution coefficients or interaction coefficients of glycerides in SC-CO<sub>2</sub> at various conditions.

In this paper, we describe the fractionation of glycerides with respect to their vapor pressure and molecular weight. Glyceride mixtures of mono-, di- and triglyceride were selected for this study since they are readily available and can be used to study the influence of molecular weight, polarity and fatty-acid composition on the fractionation of glycerides. This study identifies the important process variables, and optimizes conditions which allow the attainment of high separation efficiency.

## 2. Experimental

### 2.1. Materials

The feed material used for this study was a mixture of mono-, di-, and triglycerides called Myvatex Mighty Soft<sup>™</sup> Softener, obtained from Eastman Chemical Company (Kingsport, TN).<sup>1</sup> This material was approximately 40% monoglyceride, produced by glycerolysis of partially hydrogenated fat. It is a solid at room temperature. The composition of the feedstock, analyzed by supercritical fluid chromatography (SFC), is shown in Table 1.

Table 1  
Feed material composition<sup>a</sup>

Parameter	Weight percent		
	MAG	DAG	TAG
Mean	48.7	46.3	5.0
Standard deviation	2.9	2.5	0.4
Coefficient of variation	5.8	5.4	9.3

<sup>a</sup>Average of 13 determinations.

<sup>1</sup>Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others which may also be suitable.

The fatty acid profile of the feedstock was determined by gas chromatography of the transesterified methyl esters [31], and is shown in Table 2. The welding-grade carbon dioxide used in the experiments was obtained from National Welding Supply (Bloomington, IL) in 25 kg gas cylinders.

## 2.2. Column fractionation process

The supercritical fluid fractionations were performed in a packed column along which a temperature (density) gradient was maintained. The pilot-scale column included a preheater and four separately heated zones, each having an internal diameter of 1.43 cm and a height of 41.5 cm. A schematic diagram of the packed column supercritical fluid fractionation system is shown in Fig. 1. The column has a total height of 164 cm and volume of 260 cm<sup>3</sup>. The column utilized a protruded stainless-steel packing (0.16 in Pro-Pak, Scientific Development Company, State College, PA) which provided 94% void volume in the column. Each zone was heated to the desired temperature using Glas Col heating mantles (Glas Col Inc., Terre Haute, IN) and the temperature was recorded by Type-J thermocouples attached to the column wall. The wall temperature for each zone was maintained at  $\pm 1^\circ\text{C}$  and independently controlled (Model CN4600, Omega Engineering Inc., Stamford, CT). Two thermocouples were inserted into the flowing fluid at the top and bottom of the column to monitor the temperature of the fluid.

The column was allowed to equilibrate thermally for about 1 h before the feed was introduced. The feed mixture was melted and a liquid metering pump (Model MS-188, Haskel Inc., Burbank, CA)

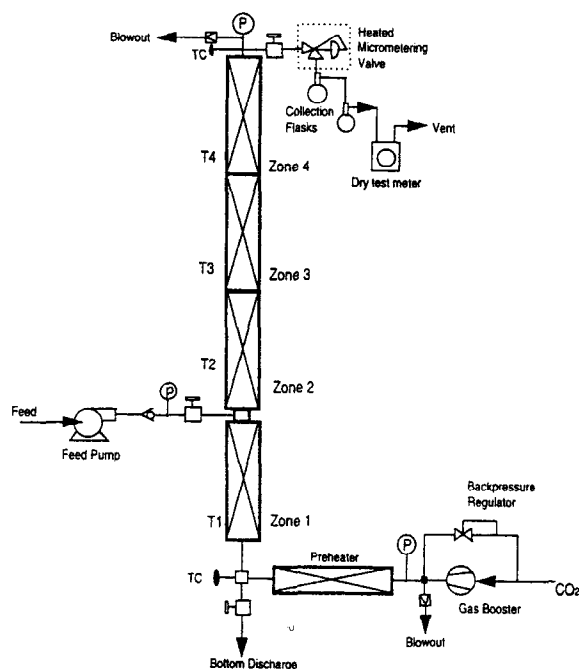


Fig. 1. Schematic diagram of the packed-column fractionation system.

connected to a stroke counter controller was used to pump feed into the column above the first heated zone. The feed line was also heated to prevent solidification of the feed mixture. For each run, 70 ml of feed was pumped in; an amount equal to the void space in zone 1. Carbon dioxide was introduced from a commercial cylinder through a filter at about 60 bar to a Haskel gas booster pump (Model ACT 62/152, Haskel Inc., Burbank, CA; capacity of 690 bar and 240 cm<sup>3</sup> min<sup>-1</sup>). The pressurized column was then equilibrated for more than 1 h before the outlet valve was opened.

As shown in Fig. 1, carbon dioxide enters the preheater and then passes through the column upward along the temperature gradient. The solute-loaded SC-CO<sub>2</sub> flows up the column through the zones of increasing temperature. The increase in temperature decreases the CO<sub>2</sub> density, allowing the higher vapor-pressure monoglycerides to concentrate at the top of the column. The fluid leaving the column was expanded across a micro-metering valve (Model 30VRMM4812, Autoclave

Table 2

Fatty acid composition (wt.%) of the feed material

Fatty acid	Composition (wt.%)
C12:0	0.7
C16:0	6.2
C18:0	23.3
C18:1	58.8
C18:2	5.6
C20:0	0.3

Engineers Inc., Erie, PA), allowing the fractionated product to be collected in a flask. Finally the gas stream passed through a dry test meter (Singer Model DTM-115, American Metering Division, Philadelphia, PA) to measure the total gas volume, and was then vented to the atmosphere.

Most runs took approximately 2 h and the total amount of top product collected was about 1 g. Short run-times and product collection times were chosen to ensure that the feed material composition would not be altered significantly during the run. At the end of each run, the column was depressurized by venting out the CO<sub>2</sub> and draining the lipid phase. The column was then cleaned in flowing CO<sub>2</sub> at 275 bar for more than 4 h.

### 2.3. Experimental design

The experiments were designed to study the effect of process parameters and the composition of the feedstock on the separation and yield of the fractionated product. The process parameters studied were the temperature gradient, pressure (density), solvent flow rate and feed composition. Experiments were conducted at pressures ranging from 172 to 345 bar, which encompasses a density range of 400–750 kg m<sup>-3</sup> at the average column temperature. An expanded CO<sub>2</sub> flow rate from 2 to 12 l min<sup>-1</sup> allowed retention times for the SC-CO<sub>2</sub> in the column of 55 to 7 min. Various pressures and temperature-gradient profiles were tested to create a density gradient as large as 420 kg m<sup>-3</sup> in the column. Each run was duplicated, and four samples were collected during each run. The amount of top product and solvent used were measured, allowing the compositions of the top product and feed to be determined.

### 2.4. Analysis of fractionated products

The composition of the top and bottom products were analyzed using a Lee Scientific Series Model 600 supercritical fluid chromatograph (Dionex Inc., Salt Lake City, UT). The internal standard used was docosane (C<sub>22</sub>H<sub>46</sub>). About 10 mg of docosane and 100 mg of the sample were dissolved in 10 ml anhydrous ether (J.T. Baker, Phillipsburg, NJ). The mixture (10 µl) was then injected into

the supercritical fluid chromatograph, which was equipped with a timed injector (200 nl injection loop; Valco Inc., Houston, TX) which was held open for 1.8 s, a Dionex SB-Octyl-50 capillary column (10 m × 50 µm i.d., 0.5 µm film thickness), a flame ionization detector operating at 350°C, and an integrator (Data Jet-CH2, Spectra-Physics, San Jose, CA). The column temperature was held at 100°C for 5 min and then increased to 190°C at a rate of 8°C min<sup>-1</sup>. The carrier gas was CO<sub>2</sub> (SEC/SFE grade, Air Products, Allentown, PA). The pressure programming used was 120 bar for 5 min, followed by an increase to 304 bar at a rate of 8 bar min<sup>-1</sup> and a final hold of 3 min. Internal standard calculations were performed to calculate the weight percentages of FFA, MAG, DAG and TAG fractions in the top and bottom products.

## 3. Results and discussion

The properties of the components in the feed material are shown in Table 3. As noted previously, there is a dramatic difference in the molecular weights of the glyceride species, suggesting that fractionation based on this parameter should be possible. Solubility parameters associated with the varying chain lengths of mono-, di- and triglyceride components decrease with increasing carbon-chain length, a change commensurate with increasing the non-polar nature of the molecular structure [33]. The differences in solubility parameters associated with mono-, di- and triglyceride species are significant. The trend to higher cohesive energy densities as one goes from tri- to di- to monoglyceride correlates with a significant increase in the polarity of glyceride species with deacylation of the parent glycerol structure. Vapor pressure is another factor which plays a significant role in fractionating glyceride mixtures. Unfortunately, published vapor pressure data, even at low pressures, for glyceride species are limited, but some available values are shown in Table 3 [32].

The results of a typical separation which can be achieved with SFF is demonstrated in Fig. 2, where the chromatograms of the feedstock and top product are shown. It can be seen that the total TAGs and DAGs are dramatically lower in the top

Table 3

Molecular weight, solubility parameter and vapor pressure [32] of mono-, di- and triacylglycerols

Fatty acid groups	TAG			DAG			MAG		
	MW	$\delta^a$ (cal <sup>1/2</sup> cm <sup>-3</sup> )	$P^{vap\ b}$ ( $\times 10^8$ )MPa	MW	$\delta$ (cal <sup>1/2</sup> cm <sup>-3</sup> )	$P^{vap\ b}$ (MPa)	MW	$\delta$ (cal <sup>1/2</sup> cm <sup>-3</sup> )	$P^{vap\ b}$ ( $\times 10^3$ )MPa
C12:0	639	8.93	4500	456	9.68	NA	274	11.37	7
C14:0	722	8.91	NA	512	9.09	NA	302	10.61	NA
C16:0	807	8.86	76.9	440	9.30	NA	266	10.44	4
C18:0	890	8.83	3.8	624	9.22	NA	328	10.17	1.4
C18:1	884	8.85	NA	620	9.33	NA	356	10.92	1.5
C18:2	878	8.87	NA	616	9.27	NA	354	10.24	NA

<sup>a</sup>Approximated using Fedors' group contribution method [33].<sup>b</sup>Vapor pressure at 190°C.

NA = not available.

product than in the feed material. The maximum deviation between two runs was less than 8.9% for the total concentration of MAGs, and less than 21.3% for the total yield collected. These recorded differences between the two runs are probably due to non-attainment of the final equilibrium solubility of the solutes in the SC-CO<sub>2</sub>. The mono-glyceride mixtures made from oils containing unsaturated fatty acids using conventional techniques are frequently exceedingly dark. The top product from the fractionation column was much lighter in color (white). The removal of the color bodies by SCF fractionation is highly desirable if the top product is to be used as a food additive, since lighter color is a valuable attribute.

### 3.1. Effect of pressure

The effect of pressure was examined by keeping the column temperature gradient constant for the four zones at 65, 75, 85 and 95°C, respectively, and a CO<sub>2</sub> flow rate of 2 l min<sup>-1</sup> (measured at 23°C and 1 atm). It has long been known that higher pressure results in an increase in the solvent power and in the solubility of glycerides. As the pressure increased the MAGs concentration in the top product dropped from 89% MAGs at 180 bar to 58% MAGs at 345 bar (Fig. 3). As the pressure increases at constant temperature, the solubility of components having lower volatilities (i.e. di- and triglycerides) increases more than the solubility of the more volatile compounds [29]. Therefore, such

an increase in solvent strength makes the process less selective. A similar effect has been observed by other researchers [13,23,30]. The increase in pressure also resulted in a higher top-product yield. The top product yield decreased from 15 g kg<sup>-1</sup> CO<sub>2</sub> to 2 g kg<sup>-1</sup> CO<sub>2</sub> as the pressure decreased from 345 to 172 bar (Fig. 4). A further decrease in pressure lowered the yield significantly, to the extent that at pressures below 152 bar there was no measurable yield collected. At pressures equal to or below 155 bar, the solubilities of glycerides are very low.

### 3.2. Effect of the temperature gradient

Determining the most effective temperature gradient along the column was done at a pressure of 207 bar and a CO<sub>2</sub> flow rate of 2 l min<sup>-1</sup>. A pressure of 207 bar gave high selectivity and a sufficient amount of yield for analysis. The low flow rate allows retention times for the solvent of more than 5 min in each zone, and ensures that adequate thermal equilibration is maintained within the column. Five different types of temperature profiles were selected for this study. Fig. 5 shows the various temperature gradient profiles which were established along the column so that  $T_4 \geq T_3 \geq T_2 \geq T_1$ . Two constant-temperature profiles were used, one at 65°C (TG-1) and the other at 90°C (TG-5). Two linear temperature gradients were tested, one using a temperature range of 65–95°C (TG-3) and the other from 50 to 100°C.

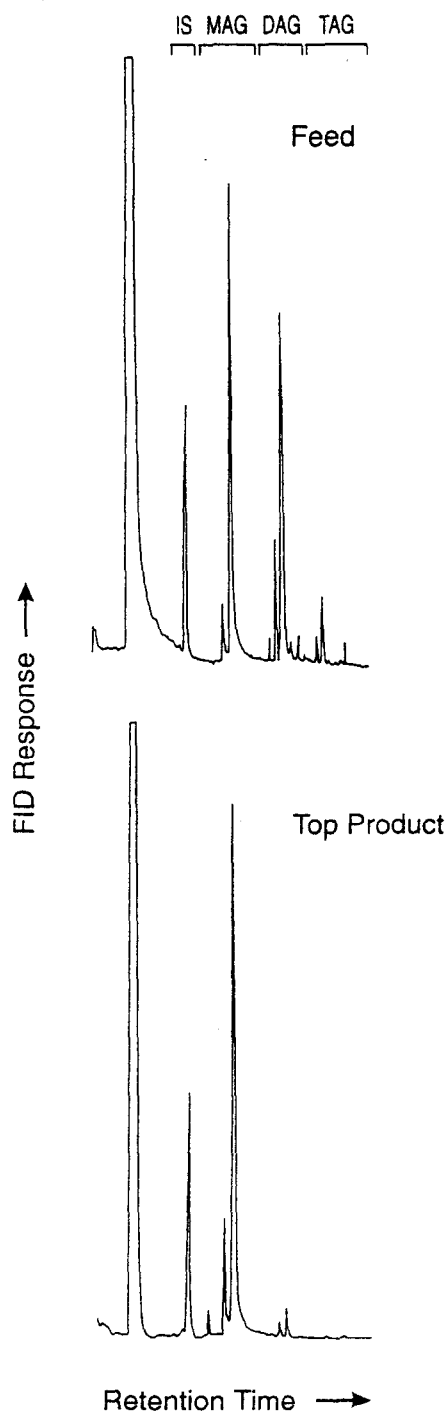


Fig. 2. Supercritical fluid chromatograms of glycerides: (a) feed and (b) top product.

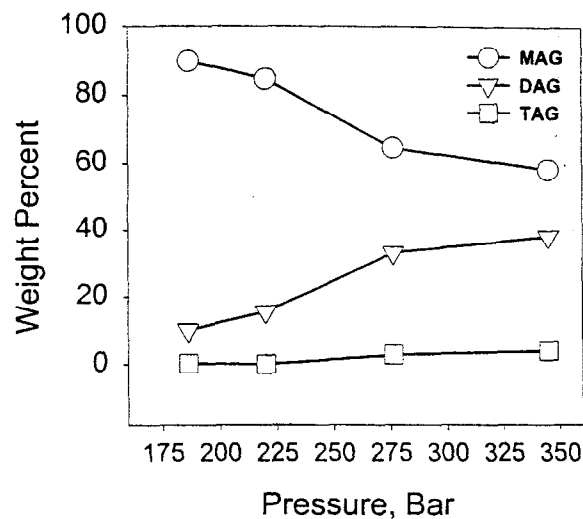


Fig. 3. Influence of pressure on the top-product composition for a column kept at a  $\text{CO}_2$  flow rate of  $3 \text{ l min}^{-1}$  and a linear temperature gradient of  $65\text{--}95^\circ\text{C}$ .

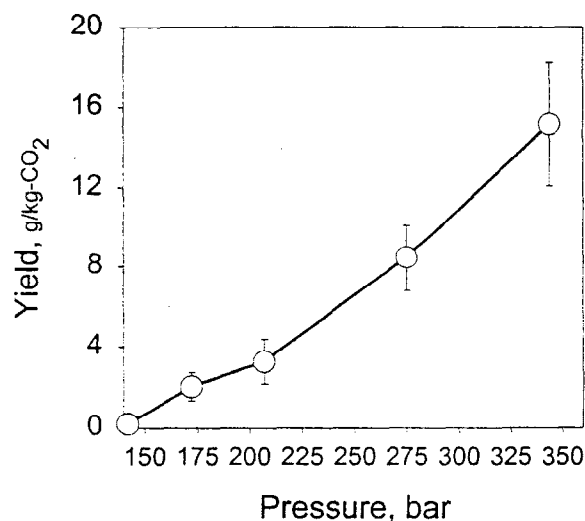


Fig. 4. Influence of pressure on the top-product yield at a  $\text{CO}_2$  flow rate of  $3 \text{ l min}^{-1}$  and a linear temperature gradient of  $65\text{--}95^\circ\text{C}$ .

Gradients TG-4 and TG-5 included two non-linear profiles, as shown in Fig. 5.

The results from using the different profiles on the fractionation of glycerides are shown in Fig. 6. A constant lower temperature ( $65^\circ\text{C}$ ) produced the least enriched product (61.5% MAGs), whereas

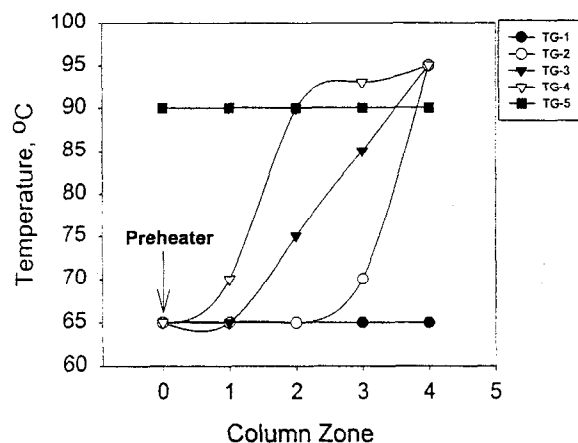


Fig. 5. Types of temperature gradient profiles tested (zone 0 is the preheater).

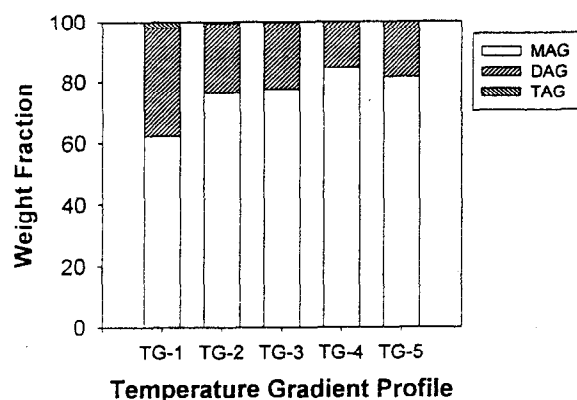


Fig. 6. Effects of temperature profiles on the top-product composition at a  $\text{CO}_2$  flow rate of  $3 \text{ l min}^{-1}$  and a pressure of 207 bar.

a constant column temperature of  $95^\circ\text{C}$  produced a MAG concentration of 81.5%. Linear temperature gradient TG-3 gave an average MAGs content of 77 and 81.8%, respectively. Therefore, the wider the temperature gradient, the better the separation using columns of the same height; this is due to the formation of an internal reflux. The other two temperature gradients (TG-2 and TG-4) produced enrichments of 76.1 and 84.6% MAGs, respectively. The top-product yield for the different temperature-gradient profiles is shown in Fig. 7. Constant temperature ( $90^\circ\text{C}$ ) appears to give a

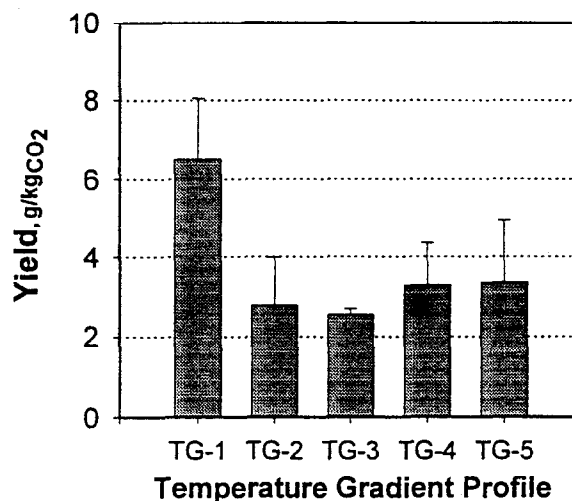


Fig. 7. Effects of temperature profile on top-product yield at a  $\text{CO}_2$  flow rate of  $3 \text{ l min}^{-1}$  and a pressure of 207 bar.

better top-product yield than the other column temperature profiles.

### 3.3. Effect of $\text{CO}_2$ flow rate

In order to examine the effect of flow rate, the fractionation column was maintained at 207 bar and a linear gradient of  $65\text{--}95^\circ\text{C}$  was utilized. As the expanded  $\text{CO}_2$  flow rate was increased from 2 to  $11 \text{ l min}^{-1}$ , there was no observed change in the top-product composition (Fig. 8). Increasing the  $\text{CO}_2$  flow rate improved the heat and mass transfer within the system, since the flowing fluid temperature approached the wall temperature more quickly. However, at high flow rates, column equilibrium was adversely affected. For example, as the  $\text{CO}_2$  flow rate increased from 1.8 to  $10.3 \text{ l min}^{-1}$ , the top-product yield decreased from 3.1 to  $1.1 \text{ g kg}^{-1}$  (Fig. 9).

### 3.4. Effect of feed composition

The effect of feed composition on the extent of enrichment was studied using two different materials (feed A and feed B) which had a MAG content of 48.74 and 63.03%, respectively. As the feed MAG concentration increased from 48.74 to 63.03%, for fractionation at 220 bar and a linear



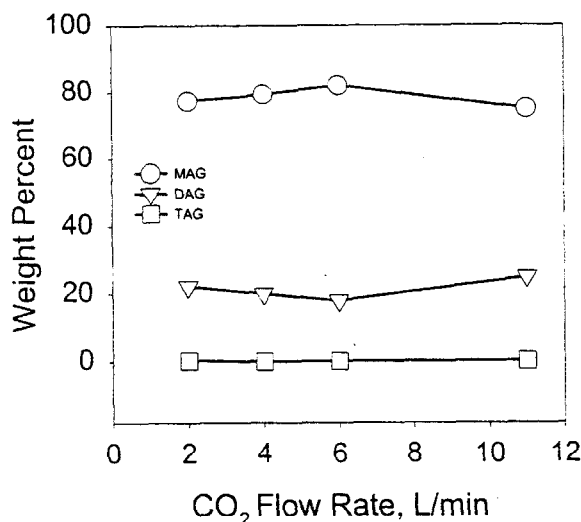


Fig. 8. Effects of flow rate on top-product composition at 207 bar and a temperature gradient of 65–95°C.

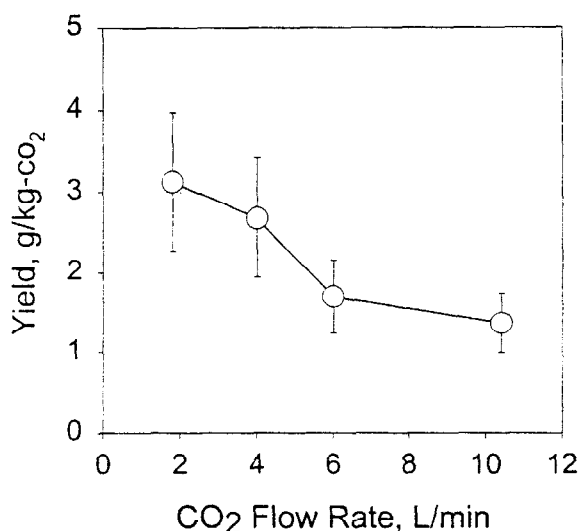


Fig. 9. Effects of flow rate on top-product yield at 207 bar and a temperature gradient of 65–95°C.

temperature gradient of 65°C and 95°C, the MAG content of the top product increased from 73.9 to 82.36% (Table 4). This result indicates that increasing the column height or employing multi-column fractionation, (i.e. by taking the top product and feeding it to a second column), it is possible to obtain a product with an even higher MAG content.

Table 4

Mean and standard deviation of feed and top-product compositions obtained at 240 bar and a temperature gradient of 65–95°C

Material	MAG (wt.%)	DAG (wt.%)	TAG (wt.%)
Feed A	48.7±2.9	46.3±2.5	5.0±0.4
Product A	73.9±5.3	28.9±7.1	0.4±0.2
Feed B	63.0±0.3	3.3±0.2	3.6±0.1
Product B	82.4±1.8	17.0±1.8	0.0

#### 4. Conclusion

Utilization of a packed column kept under a longitudinal thermal gradient employing supercritical carbon dioxide as a solvent has been shown to be effective for fractionating glyceride mixtures. MAG concentrations as high as 90 wt.% can be obtained by optimizing the separation conditions. The best enrichment conditions were found to be a pressure of 207 bar combined with a linear temperature gradient of 65–95°C on the column. Supercritical fluid fractionation of the described glyceride mixtures appears to be sensitive to pressure but not to solvent flow rate.

Other process variables such as the addition of a cosolvent to CO<sub>2</sub> could enhance the supercritical fluid fractionation of glycerides [34]. This variable, and the continuous, countercurrent operation of a thermal gradient fractionation tower under supercritical fluid conditions, are currently being investigated in our laboratory for the enrichment of glyceride mixtures derived from a synthetic process conducted in supercritical media [35,36].

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#### References

- [1] H. Birnbaum, The monoglycerides: manufacture, concentration, derivatives and applications, *Bakers Digest* 55 (1981) 6.

- [2] V.J. Krukoniš, Processing with supercritical fluids, in: B.A. Charpentier, M. Sevenants (Eds.), *Supercritical Fluid Extraction and Chromatography*, ACS Symposium Series 366, American Chemical Society, Washington, DC, 1988.
- [3] E. Stahl, W. Schilz, E. Schütz, E. Willing, A quick method for the microanalytical evaluation of the dissolving power of supercritical gases, in: G.M. Schneider, E. Stahl, G. Wilke, (Eds.), *Extraction with Supercritical Gases*, Verlag Chemie, Deerfield Beach, FL, 1980.
- [4] K.M. Scholsky, K.M. O'Connor, C.S. Weiss, V.J. Krukoniš, Characterization of copolymers fractionated using supercritical fluids, *J. Appl. Polym. Sci.* 33 (1987) 2925.
- [5] M. Daneshvari, E. Gulari, Supercritical-fluid fractionation of poly(ethylene glycols), *J. Supercrit. Fluids* 5 (1992) 143.
- [6] J.A. Pratt, M.A. McHugh, Supercritical-fluid fractionation of poly(ethylene-co-acrylic) acid, *J. Supercrit. Fluids* 9 (1996) 61.
- [7] J.A. Pratt, S.H. Lee, M.A. McHugh, Supercritical fluid fractionation of copolymers based on chemical composition and molecular weight, *J. Appl. Polym. Sci.* 49 (1993) 1493.
- [8] W.B. Nilsson, Gauglitz E.J., Jr., J.K. Hudson, V.F. Stout, J. Spinelli, Solubilities of methyl oleate, oleic acid, oleyl glycerols, and oleyl glycerol mixtures in supercritical carbon dioxide, *J. Am. Oil Chem. Soc.* 68 (1991) 2.
- [9] S.S.H. Rizvi, R.R. Chao, Y.J. Liaw, Concentration of omega-3 fatty acids from fish oil using supercritical carbon dioxide, in: B.A. Charpentier, M. Sevenants (Eds.), *Supercritical Fluid Extraction and Chromatography*, ACS Symposium Series 366, American Chemical Society, Washington, DC, 1988.
- [10] H. Hammam, B. Sivik, Fractionation of gluten lipids with supercritical carbon dioxide, *Fat Sci. Technol.* 93 (1991) 3.
- [11] R.R. Chao, S.J. Mulvaney, H. Huang, Effects of extraction and fractionation pressures on supercritical extraction of cholesterol from beef tallow using supercritical CO<sub>2</sub>, *J. Am. Oil Chem. Soc.* 70 (1993) 139.
- [12] A.R. Bhaskar, S.S.H. Rizvi, J.W. Sherbon, Anhydrous milk fat fractionation with continuous countercurrent supercritical carbon dioxide, *J. Food Sci.* 58 (1993) 4.
- [13] W. Eisenbach, Supercritical fluid extraction: a film demonstration, *Ber. Bunsenges. Phys. Chem.* 88 (1984) 882.
- [14] S. Peter, B. Czech, U. Ender, E. Weidner, Process for preparing pure monoglycerides, pure diglycerides and/or pure triglycerides, US Patent 5 434 280, 1995.
- [15] H. Büning-Pfaue, R. Eggers, A. Bartsch, Vergleich von milchfetten aus der kristallisationsfraktionierung und einem kontinuierlichen Fraktionierungsverfahren mittels überkritischem kohlendioxid, *Fat Sci. Technol.* 91 (1989) 92.
- [16] V. Kunkare, V. Antila, Extraktion von milchfett mit überkritischem kohlendioxid, *Fat Sci. Technol.* 91 (1989) 485.
- [17] S.S.H. Rizvi, A.R. Bhaskar, Supercritical fluid processing of milk fat: fractionation, scale-up, and economics, *Food Technol.* 49 (2) (1995) 90.
- [18] P.J. Carmelo, P. Simoes, M. Nunnes da Ponte, Supercritical fluid extraction of olive oils in countercurrent extraction columns: experimental results and modelling, in: *Proceedings of the Third International Symposium on Supercritical Fluids*, vol. 2, 1994, p. 107.
- [19] D. Chouchi, D. Barth, R.M. Nicoud, Fractionation of citrus cold-pressed oils by supercritical CO<sub>2</sub> desorption, in: *Proceedings of the Third International Symposium on Supercritical Fluids*, vol. 2, 1994, p. 183.
- [20] M. Sato, M. Goto, T. Hirose, Fractionation of citrus oil by supercritical fluid extraction tower, in: *Proceedings of the Third International Symposium on Supercritical Fluids*, vol. 2, 1994, p. 83.
- [21] S. Lim, G.B. Lim and S.S.H. Rizvi, Continuous supercritical CO<sub>2</sub> processing of milk fat, in: M.A. McHugh (Ed.), *Proceedings of the Second International Symposium on Supercritical Fluids*, 1991, p. 292.
- [22] W.B. Nilsson, E.J. Gauglitz, J.K. Hudson, Supercritical fluid fractionation of fish oil esters using incremental pressure programming and a temperature gradient, *J. Am. Oil Chem. Soc.* 66 (1989) 1596.
- [23] V.J. Krukoniš, J.E. Vivian, C.J. Bambara, W.B. Nilsson, R.E. Martin, Concentration of eicosapentaenoic acid by supercritical fluid extraction: a design study of a continuous production process, in: G. Flick, R.E. Martin (Eds.), *Seafood Biochemistry: Composition and Quality*, Technomic Publishing Company, Lancaster, PA, 1992, p. 169.
- [24] S. Lim, S.S.H. Rizvi, Continuous supercritical fluid processing of anhydrous milk fat in a packed column, *J. Food Sci.* 60 (1995) 889.
- [25] J.P. Friedrich, G.R. List, A.J. Heakin, Petroleum-free extraction of oil from soybeans with supercritical CO<sub>2</sub>, *J. Am. Oil Chem. Soc.* 59 (1982) 288.
- [26] J. Chrastil, Solubility of solids and liquids in supercritical gases, *J. Phys. Chem.* 86 (1982) 3012.
- [27] M. Fattori, N.R. Bulley, A. Meisen, Carbon dioxide extraction of canola seed: oil solubility and effect of seed treatment, *J. Am. Oil Chem. Soc.* 65 (1988) 968.
- [28] H. Hammam, Solubilities of pure liquids in supercritical carbon dioxide, *J. Supercrit. Fluids* 5 (1992) 101.
- [29] G. Brunner, S. Peter, Zum stand der extraktion mit komprimierten gasen, *Ger. Chem. Eng.* 5 (1982) 181.
- [30] W.B. Nilsson, Gauglitz E.J., Jr., J.K. Hudson, V.F. Stout, J. Spinelli, Fractionation of menhaden oil ethyl esters using supercritical fluid CO<sub>2</sub>, *J. Am. Oil Chem. Soc.* 65 (1988) 109.
- [31] S.D. House, P.A. Larson, R.R. Johnson, J.W. DeVries, D.L. Martin, Gas chromatographic determination of total fat extracted from food samples using hydrolysis in the presence of antioxidant, *J. Assoc. Off. Anal. Chem. Int.* 77 (1993) 960.
- [32] D. Swern, *Bailey's Industrial Oil and Fat Products*, vol. 1, 4th ed., Wiley, New York, 1979, p. 204.
- [33] R.F. Fedors, A method for estimating both the solubility parameters and molar volumes of liquids, *Polym. Eng. Sci.* 14 (1974) 147.
- [34] S. Peter, Supercritical fractionation of lipids, in: J.W. King,

- G.R. List (Eds.), *Supercritical Fluid Technology in Oil and Lipid Chemistry*, American Oil Chemical Society, Champaign, IL, 1996, p. 65.
- [35] F. Temelli, J.W. King, G.R. List, Conversion of oils to monoglycerides by glycerolysis in supercritical carbon dioxide media, *J. Am. Oil Chem. Soc.* 73 (1996) 699.
- [36] M.A. Jackson, J.W. King, Lipase-catalyzed glycerolysis of soybean oil in supercritical carbon dioxide, *J. Am. Oil Chem. Soc.*, 74 (1997) 103.